Peer Review
Experimental Toxicology Division
N H E E R L

WATER

Evaluation of Drinking Water Contaminants for Immunotoxicity

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Introduction

The 1996 amendment of the SDWA required EPA to develop a set of standards and rules (the Microbial and Disinfection Byproducts, or M/DBP) rules to address the balance of risks associated with drinking water disinfection.

The 1996 amendment of the SDWA required EPA to publish a list of Contaminants (the Candidate Contaminant List) that are not regulated but are known or are anticipated to occur in public water systems.

Disinfectants

React with humic and fulvic acids in source water to produce disinfection byproducts, or may pose a risk when present as a residual in finished water

- Haloacids (Dibromo- and Dichloroacetic acid: DBA, DCA)
  - Carciogens: as a group, associated with immunotoxicity
- Trihalomethanes (bromodichloromethane)
  - Reproductive effects
  - Carciogens: as a group, associated with immunotoxicity
- Bromate from ozonation
- Chloroform from chlorination
- Chlorite from chlorine dioxide use as a disinfectant
- Chlorine replacements
  - Chloramine

Contaminants

Organotin stabilizers in PVC pipe leach into finished water (mono- and di-substituted methyl- and butyltins)

- Structurally similar organotins are immunotoxic in adults and neonates following chronic or short-term exposure

Objectives

Project 1

- Using NTP-like tier testing approach, evaluate representative, commonly occurring D/DBPs for adult immunotoxicity in B6C3F1 mice

Project 2

- Evaluate the effects of developmental exposure to organotins, used as stabilizers in PVC water pipe, on cellular and humoral immune function in rats

Methods

Project 1
D/DBPs Adult Immunotoxicology Screening

Cooperative agreement with NTP

Exposure: 28 day drinking water, ♀B6C3F1 mice

- Dibromoacetic acid: 125, 250, 500,1000, 2000 ppm
- Dichloroacetic acid: 125, 250, 500,1000, 2000 ppm
- Bromate: 80, 200, 400, 600, 800 ppm
- Chloroform: 2.5, 10, 25, 100, 250 ppm
- Chlorite: 0.5, 1, 5, 15, 30 ppm
- Chloramine: 2, 10, 20, 100, 200 ppm

Evaluation of Immune Function

- Antigen-specific responses
  - Antibody synthesis (sheep erythrocytes)
  - Cell mediated immunity
- Nonspecific immune function
  - Natural killer (NK) cell activity
  - Phagocytic activity of macrophages
- Enumerative data
  - Leukocyte counts
  - Phenotypic analysis of lymphocyte subsets

Luebke Laboratory

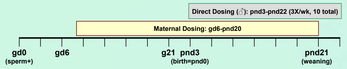
Exposure

- Bromodichloromethane
  - ♀ C57BL6mice, 28 d drinking water: 50, 250, 500 ppm
  - ♂ Rats 26 week drinking water: 70, 700 ppm
- Chlorine
  - ♀ C57BL6: 7.5, 15, 30 ppm

Evaluation of Immune Function

- Antigen-specific responses
  - Antibody synthesis (sheep erythrocytes)
  - Cell-mediated immunity
- Nonspecific immune function
  - Natural killer (NK) cell activity
  - Phagocytic activity of macrophages
- *Ex vivo* assays
  - Nonspecific responses to T and B cell mitogens

Project 2
Developmental Immunotoxicity



Exposure to 0, 2.5 or 5.0 mg DBTC/kg/dose:

Details

Timing	Exposure	Endpoint/Procedure
GD 7-PND 21	Maternal	Maternal body weight
PND 3-PND22	Direct	Body weight pups
PND 0	Maternal/Direct	Birth: pup gender & number
PND 1	Maternal/Direct	Cull litters to 4 ♂ and 4 ♀ Brain, body weights on culls
PND 21	Maternal/Direct	Wean Brain & body weights, ♀
PND 38	Maternal/Direct	Brain & body weights, ♂
PND 40	Maternal/Direct	Immunize, BSA/CFA, ♂
PND 41	Maternal/Direct	Immunize, SRBC, for IgM, ♂
PND 55	Maternal/Direct	Immunize, SRBC, for IgG, ♂

Evaluation of immune function

- Antibody synthesis
  - Immunized with SRBC after 17 (maternal) or 7 (direct) doses; IgM samples collected after 5 days
  - Boosted for IgG responses 22 days after 1^0 immunization; samples collected after 5 days
- Cell mediated immunity
  - Immunized with BSA in CFA 2 days after last DBTC exposure
  - Challenged with BSA after 7 days and evaluated 24 h later

Results

At less than toxic doses, none of the disinfectants or disinfection byproducts caused changes in immune function endpoints.

Exposure to DBTC via maternal or direct dosing did not affect the ability of offspring to mount cellular or humoral responses to injected antigen.

Maternal exposure to DBTC slowed, and direct exposure prevented normal brain weight gain at 38 days of age. Apoptosis (maternal exposure) or necrosis (direct exposure) are the likely causes of brain weight gain defects. Results of maternal dosing suggest that DBTC, or its metabolites, cross the placenta, since brain weight is typically conserved at all costs.

Conclusions and Impact

Project 1

None of the tested disinfectants or disinfection byproducts affected immune function at concentrations that far exceeded likely levels of exposure from finished drinking water. These results indicate that the immune system is not a particularly sensitive target of the tested D/DBPs.

Project 2

Although chronic exposure to DBTC causes immunotoxicity in adult rats, developmental exposure to DBTC did not affect antigen-specific immune function. Structurally similar organotins are immunotoxic in directly exposed newborn pups but have no effect on immune function if dams are exposed. On the other hand, maternal exposure to DBTC caused apoptosis and weight gain deficits in the brains of offspring, and direct dosing of pups caused necrosis and more severe weight gain deficits in offspring brains. These results suggest that the developing nervous system is more sensitive than the developing immune system to the effects of DBTC exposure.

Future Directions

Project 2: Developmental Immunotoxicology

- Evaluate mono- and dimethyltin and monobutyltin as developmental Immunotoxicants
- Determine the relative sensitivity of the developing nervous and immune systems to organotins used in the manufacture of PVC pipe
- If developmental exposure causes both immunotoxicity and neurotoxicity, conduct collaborative studies with personnel from the Neurotoxicology Division, NHEERL, to determine whether shared modes of action can be identified

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